IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Prasad DEVARAJAN et al.	:	Confirmation No: 2792
Serial No.: 10/811,130	:	Group Art Unit: 1641
Filed: March 26, 2004	:	Examiner: FOSTER, Christine E.

A METHOD AND KIT FOR DETECTING THE EARLY ONSET OF RENAL TUBULAR CELL INJURY

DECLARATION UNDER 37 CFR 1.132 OF DR. WALTER J. KEIRANS

Commissioner for Patents
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I, Dr. Walter J. Keirans, do hereby declare as follows:

1. Currently I am Division Vice President of Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL. Among other matters, I direct all of my company's renal-related research and development.

2. I hold an undergraduate degree in Zoology from the University of Connecticut and an M.S./Ph.D. in Biology from Lehigh University, Bethlehem, Pa. My area of research was the immunochemical characterization of fish egg antigens and response in population studies of pelagic species. I served as Research Scientist (3 years) in exploring antigenic selection for development of hyposensitivity vaccines (anti-allergenics) for Miles Laboratories and clinical and research product management at DuPont Biomedical (10 years) including automated clinical immunoassay development, automated DNA sequencing system chemistries and gene transfection systems for crop plant genetics. Finally, I have served in various research management positions for R&D (18 years) of blood banking immunoassay systems, hematology systems and core research of disease state biomarkers of both traditional and novel biomarkers in stratgic disease states of interest to Abbott Diagnostics.

3. I have been conducting or overseeing research in the field of renal disease, and other fields including cardiovascular disease, reproductive health, endocrinology, infectious diseases and molecular biology/DNA sequencing since 1980. The goal of this research has been, and continues to be, advancing diagnosis and treatment options by providing quality, improved, and/or novel immunoassays.

4. Abbott is the exclusive licensee of U.S. Application No. 10/811,130 ("subject application") from the assignees CHILDREN'S HOSPITAL MEDICAL CENTER, through its operating division, the Cincinnati Children's Research Foundation ("CCRF"), and THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK ("Columbia").

5. I am familiar with the subject application and its course of prosecution to date, and have reviewed the Office Action dated June 9, 2009 marked "final" and the references cited therein. I also am familiar with the presently-amended and prior claims of the subject application, and prior rejections. I am aware of the current rejection in the Office Action of the claims based on alleged obviousness. I respectfully disagree with this rejection for at least the reasons and reasoning set forth below.

6. In particular, I am aware that the following obviousness rejections have been applied in the most recent Office Action:

(a) In paragraph 16 of the Office Action (pages 6-15), the pending claims 4-5, 9-11, 33, 35, 37, 55 and 66 are rejected under 35 USC 103(a) as being obvious over (1) Matthaeus et al., "Acute Ischemic Renal Failure Induces Expression of Neutrophil Gelatinase-Associated Lipocalin and Matrix Metalloproteinase-9 in Damaged Tubuli" *Kidney Blood Press Res* (2001), Vol. 24, page 342, abstract No. P268 ("Matthaeus 1") or Matthaeus et al.,"Co-Regulation of Neutrophil Gelatinase-Associated Lipocalin and Matrix Metalloproteinase-9 in the Postischemic Rat Kidney" J. Am Soc Nephrol Vol. 12, September 2001, Pathophysiology of Renal Disease, pp. 787A ("Matthaeus 2") in

view of Ramsden et al. (US 4,640,909), Blaser et al. ("A sandwich enzyme immunoassay for the determination of neutrophil lipocalin in body fluids" Clin Chim Acta. 1995 Mar 31; 235(2): 137-45), Moses et al. (US 7,153,660), David et al. (US 4,376,110) and Muramatsu (Kidney International, Vol. 62 (2002), pages 1601-1610), or in the alternative over Matthaeus 1 or 2 and Ohlsson et al. ("Increased circulating levels of proteinase 3 in patients with anti-neutrophilic cytoplasmic autoantibodies-associated systemic vasculitis in remission" Clin Exp Immunol. (available online February 28, 2003) 131(3):528-35) in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Muramatsu.

(b) In paragraph 17 of the Office Action (pages 15-16), claims 4-5, 9-11, 33, 35, 55 and 66 are rejected under 35 U.S.C. 103(a) as being unpatentable over Matthaeus 1 or 2 in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Sanicola-Nadel et al. (US 6,664,385).

(c) In paragraph 18 of the Office Action (pages 17-18), claim 2 is rejected under 35 U.S.C. 103(a) as being unpatentable over Matthaeus 1 or 2 in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Muramatsu et al.; or in the alternative over Matthaeus 1 or 2 and Ohlsson et al. in view of Ramsden et al., Blaser et al., Moses et al., David et al. and Muramatsu et al.; or in the alternative over Matthaeus 1 or 2 in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Sanicola-Nadel et al. as applied to claim 66 above, and further in view of either one of Valkirs et al. (US 2003/0109420 AI) or Linzer et al. (US 3,635,091).

(d) In paragraph 19 of the Office Action (page 18-19), claim 55 is rejected under 35 U.S.C. 103(a) as being unpatentable over Matthaeus 1 or Matthaeus 2 in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Muramatsu et al.; or in the alternative over Matthaeus 1 or Matthaeus 2 and

Ohlsson et al. in view of Ramsden et al., Blaser et al., Moses et al., David et al. and Muramatsu et al.; or in the alternative over Matthaeus 1 or 2 in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Sanicola-Nadel et al. as applied to claim 66 above, and further in view of Kosako et al. (US 5,527,714).

(e) In paragraph 20 of the Office Action (pages 19-21), claim 60 is rejected under 35 U.S.C. 103(a) as being unpatentable over Matthaeus 1 in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Muramatsu et al.; or in the alternative over Matthaeus 1 and Ohlsson et al. in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Muramatsu et al.; or, in the alternative, over Matthaeus 1 in view of Ramsden et al., Blaser et al., Moses et al., David et al., and Sanicola-Nadel et al., all as applied to claim 66 above, and further in view of Zanardo et al. ("Acute renal failure in the patient undergoing cardiac operation. Prevalence, mortality rate, and main risk factors," J Thorac Cardiovasc Surg. 1994 Jun; 107(6): 1489-95).

7. I am familiar with the characterization in the Office Action of the teachings in the prior art and the differences between the prior art and the claims at issue. Some of these arguments are summarized as follows:

(a) In paragraph 16 (pages 7-8) the Office Action asserts that Matthaeus 1 teaches that levels of NGAL protein are upregulated in response to ischemic renal injury in a rat model (citing entire selection) wherein by contrast, control animals displayed only minor expression of NGAL, alleging a demonstration that renal injury and repair is associated with an upregulation of NGAL. The Office Action states that Matthaeus 1 further reports that NGAL was elevated "after 24 and 48 hours" of renal ischemia as assessed by Western blot analysis. The Office Action asserts that Matthaeus 2 teaches that NGAL protein expression was upregulated after ischemic injury in a rat model of renal ischemia, alleging a demonstration that upregulation of NGAL is associated with renal injury as well as repair (citing

entire selection). The reference purportedly further teaches that NGAL may play a critical role in the renal response to ischemic injury (based on the last sentence of this reference). The Office Action says that the Matthaeus references differ from the claimed invention in that Matthaeus 1 and 2 fail to specifically teach detecting NGAL in urine as claimed.

(b) Paragraph 16 (page 8) continues that "It was well known in the art that disease processes may produce changes in the levels of certain specific analytes, and that measurement of the levels of such analytes can be used to detect the presence of the disease. The Office Action takes this as admitted prior art because applicant purportedly has failed to traverse this assertion. Based on this, the Office Action asserts that it would have been obvious to one of ordinary skill in the art to detect NGAL for the purpose of diagnosing acute renal injury in light of the teachings of Matthaeus 1 or Matthaeus 2 that NGAL is specifically elevated in this disease condition, and further in view of the general knowledge of one skilled in the art that markers changed in response to disease can be used as biomarkers for diagnosis of the disease.

(c) Paragraph 16 (pages 7-8) also states that Matthaeus 1 and 2 make clear that the rat studies were performed as an animal model of disease. The Office Action adds that "Matthaeus 1 state that the purpose of their experiment is to 'further elucidate the processes involved in renal injury and repair'"and that the "findings reported therein support a 'critical role in the renal response to injury' for NGAL," and correlate upregulation of NGAL with ischemic renal tubular cell injury. The Office Action continues that given such a teaching, it would have been obvious to detect NGAL in human subjects for the clear benefit of diagnosing human disease and that NGAL could be used as a biomarker of renal ischemia.

(d) In paragraph 16 (pages 8-9), the Office Action continues that in such a case, it would have been further obvious to employ urine as the sample source, rather than the kidney tissue samples examined in the rat model of Matthaeus 1 or 2 because one skilled in the art would immediately recognize that isolation of kidney tissue would be very invasive, and therefore an unsuitable method for diagnosing renal injury in humans. The Office Action continues that alternative sources of samples for biomarker detection were known in the art; specifically, it was well known to one skilled in the art of biochemical assay at the time of the invention that urine is a non-invasive and easily collected type of sample (citing, as example Ramsden et al., column 1, lines 15-16, for its purported teaching that urine samples are noninvasive and convenient, and that urine is an easily collected and non-invasive sample source for assay of biological analytes). The Office Action states that it would have been obvious to use urine as the sample source instead of the kidney tissue samples when detecting NGAL for diagnosis of renal injury in human subjects, for the advantage of being a non-invasive and easily collected sample.

(e) The Office Action further asserts in paragraph 16 (pages 9-10) that one would have a reasonable expectation of success because it was known in the prior art that NGAL is excreted in urine, as taught by Blaser et al. and Moses et al. The Office Action states that Blaser et al. teach detection of human neutrophil lipocalin (NGAL) in urine by sandwich ELISA (citing, in particular, the abstract; page 139, section 2.4; and pages 142-143, sections 3.3-3.4), and that Moses et al. also teach that NGAL may be detected in human urine by Western Blot (citing Example 2 and Figure IB). The Office Action says that in light of the teachings of Blaser et al. and Moses et al., one skilled in the art would have a reasonable expectation of success in using urine as a sample source for detection of NGAL in response to renal injury (rather than kidney tissue as taught by Matthaeus 1 and 2) since NGAL was known to be excreted in urine.

8. Among other disagreements with this characterization set forth in paragraph 7 immediately above, I respectfully disagree with the characterization in the Office Action of the aforementioned teachings in the prior art (in terms of scope and content) and the differences between the prior art and the claims at issue as follows:

(a) With regard to paragraph 7(a), I disagree with the statements in the Office Action saying that Matthaeus 1 and 2 et al. teach that NGAL levels correlate with renal function. The results set forth in abstract form in Matthaeus 1 and 2 provide insight into the appearance and changes related to biomolecules found by representational difference analysis (RDA). Experimental and control animal results are compared in which MMP9, TIMP1 and NGAL are upregulated in kidney ischemia rat models. It should be noted by definition of RDA, the statement of function cannot be addressed in these static models, particularly when controls are not the same animals tested at all time points in the experimental protocol nor are the 12 and 24 hour animals. Numbers of animals sacrificed at each test point are not declared leaving open the statistical validity of the results. Therefore, the subsequent impact of findings in individual rats on kidney function is impossible to conclude as each animal is sacrificed at stated test points – there can be no functional outcomes. Kidney failure is extrapolated from immunohistochemistry and composite results. Moreover, the results set forth in abstract form in Matthaeus 1 and 2 appear not to have published subsequently anywhere else. Thus, it is near impossible for the applicants and Examiner to understand exactly ***what*** may or may not have been uncovered by the investigators. The lack of later publication of a full report - - unusual - - calls into question exactly what the references teach or suggest.

(b) With regard to paragraph 7(b), the Office Action takes as **admitted prior art** that disease processes may produce changes in the levels of certain specific analytes, and that measurement of the levels of such analytes can be used

to detect the presence of the disease. Based on this, the Office Action asserts that it would have been obvious to one of ordinary skill in the art to detect NGAL for the purpose of diagnosing acute renal injury in light of the teachings of Matthaeus 1 or Matthaeus 2 that NGAL is specifically elevated in this disease condition, and further in view of the general knowledge of one skilled in the art that markers changed in response to disease can be used as biomarkers for diagnosis of the disease. However, these teachings, either alone or in combination with any of the other applied references, miss a logical connection between the art of record, and the invention as supplied by the subject application. Namely, ***even if*** the teachings of the prior art are accepted as characterized in the Office Action, for NGAL to function as biomarker allowing a viable NGAL assay to be obtained there is a need, amongst other things, for (a) specificity of the target molecule being assayed for the disease at issue, (b) specificity of the target molecule for the sample type being assessed, and (c) sensitivity of the assay for the target molecule being detected. For all the reasons discussed in the paragraphs below, this need was not satisfied by the prior art, but only by the applicants' invention.

(c) With regard to paragraphs 7(d) and 7(e), the Office Action states that it would have been further obvious to employ urine as the sample source, rather than the kidney tissue samples examined in the rat model of Matthaeus 1 or 2 because one skilled in the art would immediately recognize that isolation of kidney tissue would be very invasive, and that one would have a reasonable expectation of success because it was known in the prior art that NGAL is excreted in urine, as taught by Blaser et al. and Moses et al. Even though neither Matthaeus 1 or 2 nor Ohlsson examined NGAL levels in urine (Ohlsson et al. examined blood), the Office Action states it would have been obvious to use urine as the sample source instead of the kidney tissue samples when detecting NGAL for diagnosis of renal injury in human subjects, based on the advantage of being a non-invasive and easily collected sample (as taught by Ramsden et al.). However, the overwhelming majority of diagnostic tests that measure proteins use blood as

the matrix. Urine is rarely the diagnostic media of choice. The problem with urine is that even if a protein is observed in the urine (whether made by the tubules or present during filtration), the impact of absorption by the tubules en route to making urine can make the urine concentration of the protein diagnostically useless. In other words, the mere presence of NGAL in the urine does not make it useful. Furthermore, as discussed in 9 below, absent the applicants' invention, there was no apparent specificity of NGAL for either renal disease - - much less for renal tubular cell injury - - or for urine. It should be emphasized that while the Office Action relies on Moses et al. to support use of urine as an appropriate biological specimen in assessing NGAL, the reference more prominently discloses both free forms and complexes of NGAL with MMP9 in urine – and the conclusion that this provides evidence for NGAL modulating MMP9 activity in cancer patients. MMP9 is known to directly play a role in remodeling and repair functions (Moses et al.) and Matthaeus et al. state MMP9 and NGAL can form covalent disulfide complexes. An alternative conclusion from the combined references is that MMP9 is serving it's known role of remodeling and repair functions in acute ischemic renal failure, and that upregulation and expression of NGAL is a feedback mechanism imposed as a regulatory function of NGAL on MMP9 to prevent it's in vivo degradation. Experimental designs in the cited references are incapable of isolating and defining the role of NGAL in acute kidney injury that is identified in the instant invention.

9. Additionally, I respectfully disagree with the characterization in the Office Action of the invention as obvious over the prior art based on the following evidence:

(a) Prior to the invention of the subject application, there had been relatively little success in identifying biomarkers that could be used at an early stage for an assay for acute renal tubular cell injury. Whereas markers that can assess renal function later than 24 hours of the onset of renal injury and after are

available, particularly serum or plasma creatinine, a urine assay for detecting renal tubular cell injury (RTCI) within 24 hours of a suspected renal injury was not. Such an assay of RTCI requires a biomarker that (a) increases or changes in value at less than 24 hours, (b) is specific for the sample type being assessed (i.e., urine), (c) is specific for disease (i.e., renal disease) or preferably source of disease (i.e., renal injury) being assessed, and (d) is sensitive for the target molecule (i.e., NGAL) being detected.

(b) In the context of a renal assay, finding a biomarker that increases in value at within less than 24 hours of a suspected RTCI is particularly difficult. Prior to the invention of the subject application, others had tried to identify such a marker (see, e.g., Muramatsu et al., cited in the Office Action and published after the Matthaeus et al. references). Cyr61 was a secretory protein demonstrated by Muramatsu et al. to be upregulated in rat ischemia model and secreted into urine using RDA, northern and southern blots and immunohistochemistry (essentially same experimental approaches as Matthaeus et al.). Cyr61 was analyzed at 42 kD and it was noted that several non-specific higher molecular weight bands were also found. Although a specific function for Cyr61 was not determined, it was present in urine within 3-6 hours and peaked at 6-9 hours following renal injury. It is surprising that if detecting NGAL in urine rapidly following renal injury was obvious to one of ordinary skill, that Muramatsu et al. failed to identify it in the high molecular weight fraction of urine in their work published after Matthaeus et al. They also did not discuss NGAL nor did they cite Matthaeus et al. in their paper.

(c) In view of failure to identify other renal biomarkers that could be effectively applied to assess RTCI within a timeframe of less than 24 hours, at the time of the invention of the subject application, the need for biomarkers for detecting RTCI at less than 24 hours was long-felt, but remained unsolved. An

increase in serum or plasma creatinine, detectable at 24 hours or later, was the gold standard for assessing renal function. However, by the time an increase in creatinine can be detected, as much as 50% or more of renal function may be lost. This is because the serum creatinine increase occurs much later than the renal injury resulting in the loss of function.

(d) The failures of others to identify biomarkers detectable at within 24 hours of renal injury would lead to general skepticism prior to applicants' invention regarding the use of NGAL to assess acute renal injury. Additionally, there were further more particular reasons at the time of the invention why one of ordinary skill would not consider or recognize urine NGAL for assessing RTCI within 24 hours of suspected renal injury, even in view of the teachings in the prior art.

(e) First, NGAL had been detected in tissues and sample types other than urine. NGAL from such other sources may produce a high background, or a complete lack of specificity, precluding use as a marker. At the time of the invention, NGAL mRNA and/or protein had been detected in and associated with neutrophils (see, e.g., Allen et al., Biochimica et Biophysica Acta, 991:123-133 (1989), Kjeldsen et al., Blood 83(6): 1640-1649 (1994), Kjeldsen et al., Blood 82(10): 3183-3191 (1993), and Kjeldsen et al., Blood 83(3): 799-807 (1994)), blood plasma of patients with systemic vasculatis (e.g., Ohlsson et al.) adult bone marrow, uterus, prostate, salivary gland, stomach, appendix, colon, trachea, and lung (e.g., Cowland et al., Genomics 45: 17-23 (1997)), and extracellular fluids of female reproductive tract (Costantini et al., Minerva ginecologica, 54:5, 387-92 (2002 Oct)). Prior to applicants' invention, this detection of NGAL in other than renal tissue or urine would seriously call into question whether NGAL could be assessed in urine as a sample source for kidney injury, distinct from other sources. In particular, appearance of NGAL in blood (red **or** white cells), would raise a

question about whether a useful urine NGAL measure could be derived, given the known fact that blood can get into the urine with infection of the kidneys or the bladder, or if there is inflammation due to the presence of stones, immune disorders, allergies, growths anywhere along the genitourinary system, and in many other situations.

(f) Second, at the time of the invention, NGAL had been associated with disease states other than renal disease or RTCI. Venge and colleagues (e.g., Xu et al., Scand. J. Clin. Lab Investigation, 55:125-131 (1995)) determined that serum or plasma NGAL or HNL present in respiratory distress conditions was capable of differentiating viral from bacterial-mediated infection. Moses et al. have documented free and complexed NGAL associated with MMP9 known to be present in several cancers (thyroid, ovarian, breast, colon, etc.). An increase or alteration of NGAL in response to other stimuli would cause one to question whether NGAL could be specifically associated with renal disease or RTCI, particularly within a timeframe of within 24 hours of suspected renal injury. For instance, NGAL had been detected in urine of healthy donors (e.g., Blaser et al.), and increased at the mRNA, and/or protein level with cytokine withdrawal (e.g., Devireddy et al., Science 293: 829-834, (2001)), neutrophil activation, inflammation and infection (e.g., Xu et al., Scand. J. Clin. Lab Investigation, 55:125-131 (1995)); Venge, Allergy, 49(1):1-8 (1994)), cartilage and muscle differentiation (Zerega et al., European journal of cell biology, 79:3, 165-72 (2000 Mar)), and in tumors or cancer (e.g., Bartsch et al., FEBS Lett. 357:255-259 (1995); Gould et al., US 5,627,034; Conklin, US 6,143,720; and Moses et al.). All these associations, particularly with the normal cell, inflammation and infection (which raise issues of potential high background), would call into question prior to applicants' invention whether NGAL could be specifically associated in any way with any renal disease, much less with RTCI. Indeed, upon identification of NGAL in urine of patients with renal cancer and bladder cancer, Yan et al., J. Biol. Chem., 276:37258-37265 (2001) specifically raise the

possibility of neutrophil infiltration as the source of the urine NGAL by stating in the last sentence of the third paragraph in the "Discussion" that: "However, it remains possible, that the urinary MMP-9-NGAL complex may be composed of MMP-9 and NGAL secreted by the neutrophils that have infiltrated the tumor sites."

10. For at least all the reasons in paragraphs 8 and 9 above, it is my understanding and belief that one of ordinary skill in the art at the time of the subject invention would not have been able to predict with any certainty based on the art made of record (e.g., Matthaeus 1 and 2) that one would find NGAL in the urine, much less be able to use NGAL as a biomarker in a urine NGAL renal assay. For at least all the reasons above, it is my understanding and belief that one of ordinary skill working in the field at the time of the invention and considering the teachings of the art cited in the Office Action in the context of what all else was happening in the field would have no reasonable expectation that urine NGAL could be successfully employed to assess RTCI at within less than 24 hours of a suspected renal injury.

11. For at least all the reasons in paragraphs 8 and 9 above, it is my understanding and belief that the findings in the subject application regarding the invention and showing that urine NGAL could be successfully employed to assess RTCI at within less than 24 hours of a suspected renal injury were unexpected and not predicted. This is confirmed by the guarded optimism set forth in the reference of Stefan Herget-Rosenthal (Lancet, 365, 1205-1206 (2005)), which was published in the same volume as and introduced the studies setting forth the data present in only one of the examples of the subject application.

12. Based on my understanding and belief, the applicants of the subject application were the first to recognize use of NGAL as a biomarker for assessing RTCI at within less than 24 hours of a suspected renal injury. Abbott recognized this upon first learning of the work of Doctors Devarajan and Barasch. Based on Abbott's perception at that time that the results of the inventors now set forth in the subject patent application were truly surprising, and Abbott's

perception of the usefulness of NGAL as a renal biomarker, Abbott licensed the intellectual property from the assignees CCRF and Columbia.

13. For at least all the reasons in paragraphs 7 through 12 above, I recommended that Abbott proceed with development and commercialization of the assay to satisfy an unmet diagnostic need and provide a much needed ability to assess RTCI at within less than 24 hours of a suspected renal injury.

I further declare that all statements made of our knowledge are true and that all statements made on information and belief are believed to be true; further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001 and may jeopardize the validity of the application or any patent issuing thereon.

12/09/2009
Date

Walter Keirans
Walter J. Keirans

18 USC 1001: "Whoever in any matter within the jurisdiction of any department or agency of the United States knowingly and willfully falsifies, conceals or covers up by any trick, scheme, or device a material fact, or makes any false, fictitious or fraudulent statements or representations, or makes or uses any false writing or document knowing the same to contain any false, fictitious or fraudulent statement or entry, shall be fined not more than $10,000 or imprisoned not more than five years, or both."